Indigenous Nitrogen Supply of Rice Is Predicted by Soil Organic Carbon

Efficient management of rice (Oryza sativa) nutrition across soils ranging from organic to mineral soils varies widely because of large contributions of nutrients, including N, from the indigenous supply. This study tested the hypothesis that the indigenous N supply (INS) would increase if the soil organic carbon (SOC) content of the rice paddy soil increased, evaluated across a wide range of SOC content. The INS, defined as N uptake from N omission plots, was estimated from 54 plots over a 3-yr period at two locations in the Sacramento-San Joaquin Delta over a range of SOC from 6 to 232 g SOC kg$^{-1}$. Additionally, 10 N rate trials (0 to 160 kg N applied ha$^{-1}$) were conducted concurrent with the N omission plots. The INS did not increase as SOC increased across the entire SOC gradient and, instead, exhibited a concave quadratic trend across the SOC gradient, greatest in the 110 to 170 g SOC kg$^{-1}$ range and lower in sites with less than 110 g SOC kg$^{-1}$ or more than 170 g SOC kg$^{-1}$. Consequently, positive yield response to N fertilizer was observed in soils with low INS, with no positive yield response on soils with high INS. This study indicates that the INS can be predicted by the SOC content; hence, fertilizer-N recommendations should include considerations for SOC content.

Abbreviations: INS, indigenous N supply; SOC, soil organic C; SOM, soil organic matter.
Given that SOM mineralization contributes to the INS, it is reasonable to presume that the size of the INS is linked to the amount of SOM present to mineralize and that former wetland soils, which are typically high in SOM, would have a high INS. However, earlier studies have shown a poor relationship between soil organic C (SOC), a proxy measurement for SOM, and the INS of rice paddy soils (Dobermann et al., 2003; Cassman et al., 1996a), although the range of observations was limited to soils with less than 28 g SOC kg\(^{-1}\). Non-agronomic studies of organic soils under anaerobic conditions cast further uncertainty about the connection between SOC and N mineralization; reported N mineralization rates range from net mineralization (Zanner and Bloom, 1995; Terry, 1980) to net immobilization (Schmidt et al., 1999). Agronomically and environmentally sound fertility recommendations suitable for rice cultivated on organic soils rather than the typical mineral soils depend on decreasing this uncertainty.

The Sacramento–San Joaquin Delta presents a unique opportunity to study the relationship between the INS and SOC. A variety of soils ranging from mineral to organic composition exist in a relatively small geographic area (Deverel and Leighton, 2010), allowing for rice cultivation under similar management and climate but with vastly different SOC content. Expanding the range of SOC under consideration beyond that of earlier studies allows for the detection of relatively subtle relationships between the SOC and the INS. Through field and laboratory experiments, this study sought to investigate the INS across a wide range of SOC concentrations. Specifically, it was hypothesized that the INS would be linearly proportional to the SOC content of the soil across a wide gradient and that crop yield response to added N would decrease as SOC increased.

**METHODS**

**Site Description**

Field studies were conducted from 2011 to 2013 in the Sacramento–San Joaquin Delta. A total of 10 N rate trials were established to evaluate rice grain yield response to N across a gradient of SOC from 27 g kg\(^{-1}\) to 232 g SOC kg\(^{-1}\) (trials designated hereafter as SOC-27 to SOC-232). Nine N rate trials took place on Twitchell Island (38.107314 lat, −121.654808 long, −3 m elevation): two in 2011, two in 2012, and five in 2013. All sites at Twitchell Island were within 1.75 km of each other and under management of the same farmer. The soils at the Twitchell Island site are Ridge mucky silt loams classified as euic, thermic Terric Haplosaprists, with an average of 400 g SOM kg\(^{-1}\) (roughly 210 g SOC kg\(^{-1}\)) in the top 43 cm and 0 g SOM kg\(^{-1}\) below 43 cm (Soil Survey Staff, 2013). Lower variability was observed in prestudy soil surveys at the Wright-Elmwood Tract location, with SOC in the top 15 cm ranging from 60 g SOC kg\(^{-1}\) to 170 g SOC kg\(^{-1}\) (data not shown). Both sites are located in a Mediterranean climatic zone, characterized by warm, dry summers and mild, wet winters.

Both locations followed similar management practices. Rice was established by drill seeding at 15-cm spacing between rows. Rice variety ‘M-104’ was planted in 2011–2012 and in one trial in 2013. All other trials in 2013 were planted with variety ‘M-206’. Although ‘M-104’ and ‘M-206’ are different varieties, they have similar N requirements (Mutters et al., 2013). When the rice was at the three- to four-leaf stage (approximately 1 mo after planting), a permanent flood was established until a few weeks before harvest. Weed and water management followed typical practices (Dickey, 2013), as determined by the farmer.

**Field N Rate Trials and Omission Plots**

Each N rate trial was conducted as a randomized complete block design with four replications, except SOC-176 (2013) which had three replicates. Plot sizes varied by year but ranged from 16 to 20 m\(^2\). Treatments consisted of 0, 40, 80, 120, and 160 kg N ha\(^{-1}\) (designated hereafter as 0N, 40N, 80N, 120N, and 160N, respectively) applied as urea broadcast within 5 d of permanent flood, except in SOC-176 where an irrigation malfunction resulted in N application 10 d before flood establishment. Phosphorus and K fertilizers were applied to all treatments at a rate of 21.5 kg P ha\(^{-1}\) and 83 kg K ha\(^{-1}\) at the time of N application to ensure these nutrients were not limiting.

In 2013, 15 additional N omission plots were established outside of the rate trials to provide a broader estimate of INS variability: 14 plots at the Twitchell Island study site and 1 at the Wright-Elmwood Tract study site. The N omission plot locations were dispersed at the Twitchell Island site to capture SOC and geographic variability. The study area was divided into sectors and each omission plot was randomly placed inside each sector and at least 10 m from the field border. Multiple omission plots were established in sectors containing under-represented soils (e.g., less than 23 or greater than 200 g SOC kg\(^{-1}\)). Fertilizer was omitted from each plot by covering the plot area with a tarp 10 to 20 m\(^2\) at the time of broadcast fertilizer application. Phosphorous and K were then applied by hand at 21.5 kg P ha\(^{-1}\) and 83 kg K ha\(^{-1}\) to each plot.

Soil pore-water samplers (ceramic suction cup type or Rhizon MOM 10 cm, Rhizosphere Research Products, Wageningen, The Netherlands) were installed in 2013 at each trial and at 10 omission plots (total of 16 sites) at two depths below the soil surface, 15 cm and 60 cm, before permanent flood. Each sampler was installed by extracting soil with a Dutch auger, inserting the samplers, backfilling with 10 to 15 cm of silica sand, followed by sealing the hole with 4 to 6 cm of bentonite clay to prevent preferential flow. Surface and pore water were sampled...
four times throughout the growing season at approximately evenly spaced intervals beginning 1 w after permanent flood and ending 1 w before draining for harvest. Samples were collected using acid-washed and evacuated 10 mL vials. All samples were kept on ice or refrigerated following collection and were analyzed for NH$_4^+$ and NO$_3^-$ within 24 h by colorimetric methods (Forster, 1995) analyzed on a spectrophotometer (Shimadzu UV-160, Shimadzu, Kyoto, Japan).

Soil samples were collected as a composite of six to eight cores (2-cm diameter) taken before permanent flood in each omission plot from the depth of 0 to 15 cm. Samples were air dried, ground to pass a 2-mm sieve, and ball milled. Samples were encapsulated and analyzed for soil organic C and total N by the GC combustion method (Sharp 2006). Soil pH (saturated paste) for each trial was measured on a composite of soils from the four omission plots from the 0- to 15-cm soil layer.

Trials were hand harvested once the 160N treatment reached physiological maturity, defined as grain moisture below 28%. In two cases where the 0N plots matured much earlier than the 160N plots and bird foraging was likely (SOC-27 and SOC-28), the 0N plots were harvested a week prior than the remaining treatments. The total aboveground biomass in the center area in each plot was harvested by cutting the plants at soil level in each plot was harvested by cutting the plants at soil level in each plot. After being dried at 60°C until constant weight, the total aboveground biomass was separated into straw and grain. Both straw and grain were ground, encapsulated, and analyzed for N content as described earlier for soil samples.

To characterize grain yield response to applied N, the maximum yielding responsive treatment was determined for each trial. A responsive treatment was defined as a treatment which yielded significantly greater than the preceding N treatments, when treatments were ordered from 0N to 160N. In cases where no significant N response was observed, the maximum responsive treatment was set to 0N.

**Soil Incubations**

Anaerobic lab incubations were conducted on the six soils from 2013 following Saeed (1995). In brief, soils from the three or four omission plots from each of the six trials in 2013 (Table 1) were air dried and ground to pass a 2-mm sieve, combined equally by weight, and mixed thoroughly. Seven grams of each soil was placed in acid-washed 200-mL glass bottles along with 3.25 g of KCl-saturated ion exchange resin beads (Dowex HCRW-2). Bottles were topped with 50 mL of distilled water, flushed with 0.5 mol fraction CO$_2$/0.95 mol fraction N$_2$ gas, capped tightly, and placed in an incubation oven at 25°C. Bottles of each soil were randomly assigned to be extracted for mineral N after 0, 7, 14, 28, 49, and 77 d of incubation, with three replicates for each soil at each time point. At each extraction, 50 mL of 4 mol L$^{-1}$ KCl was added to each bottle, placed on a shaker table for 1 h and filtered. Extracts were analyzed for NH$_4^+$ and NO$_3^-$ as described earlier for pore-water samples.

**Statistical Analysis**

To test the hypothesis that the INS is linearly proportional to SOC, the two alternatives of no relationship and a nonlinear relationship were tested by a mixed-effects model. Step-wise regression was conducted using SOC, SOC$^2$, C to N ratio, pore-water N concentrations, fertilizer-N applied, rice variety, and pH as predictors and block, trial, location, and year as random effects. The final model assumes that the effects of SOC and SOC$^2$ were fixed between locations and years and with a random intercept and coefficient for fertilizer-N applied by block nested in trial. The intercept in this model is interpreted to be the sum of all non-SOC contributions to the INS for each site. The coefficient for fertilizer-N applied is interpreted as the N uptake efficiency (i.e., the portion of fertilizer-N applied that was taken up by the crop). The 80N treatment plots in SOC-144 experienced suspiciously high N uptake (equivalent to roughly 90% fertilizer-N uptake efficiency and double the other treatments in the same trial [Fig. 2a]) so were excluded from all further analysis.

Mixed-effects models were fit using the R statistics program (R Core Team, 2014) and the lme4 package (Bates et al., 2014). Bootstrapped confidence intervals were computed with the 'con-
Results

Site Characteristics

Across N rate trials and N fertilizer omission plots, SOC varied from 6.1 to 232 g SOC kg$^{-1}$, with most soils falling in the range of 100 to 150 g SOC kg$^{-1}$ (Table 1). Total N covaried with SOC, and C to N ratios were between 13 and 16 except for two soils (SOC-232 and 172 g SOC kg$^{-1}$ N omission plot), which had a C to N ratio greater than 19. Soil pH varied from 4.9 to 7.1 but was not correlated with other measured soil characteristics. Surface floodwater and 15-cm pore-water N concentrations were uniformly low throughout the season (less than 0.5 mg NH$_4$-N L$^{-1}$); 60-cm pore-water N concentrations varied across sites (ranging from 0.5 to greater than 50 mg NH$_4$-N L$^{-1}$), but were not correlated with either SOC or aboveground N uptake (data not shown). In general, 60-cm pore-water N concentrations were stable throughout the season, with only two sites exhibiting increased N concentrations as the season progressed (data not shown).

Indigenous Nitrogen Supply

The INS estimated from N omission plots ranged from 50 to 260 kg N ha$^{-1}$ (Fig. 1). The mixed-effects model estimated significant linear and quadratic effects of SOC on the INS (Table 2). The INS increased as SOC increased up to the estimated inflection point of 131 g kg$^{-1}$ (Fig. 1), after which the INS decreased. At the inflection point, the estimated INS averaged 189 kg N ha$^{-1}$ (Fig. 1). The fixed effects of SOC, SOC$^2$, and fertilizer-N uptake efficiency together explained 44% of the observed variability in the INS ($R^2_{marginal}$), while the model as a whole explained 81% of the observed variability in the INS ($R^2_{conditional}$; Table 2).

Nitrogen Uptake and Yields in Response to Nitrogen Fertilization

Crop N uptake among the fertilized plots ranged from 102 to 331 kg N ha$^{-1}$ depending on soil and N fertilizer treatment (Fig. 2a). Fertilizer-N uptake efficiency, the proportion of fertilizer-N taken up by the crop as estimated from the mixed-effects model, averaged 38%. Estimated trial-level fertilizer-N uptake efficiencies (overall fertilizer-N uptake efficiency plus random effect of trial) varied between 13% and 53% (Tables 2 and 3).

Yields across all treatments and years averaged 10,100 kg ha$^{-1}$ with maximum mean yield of 13,800 kg ha$^{-1}$ observed in SOC-27 with the 160N treatment (Fig. 2b). At sites with higher INS (Fig. 1 and 2a), there was a small or negative yield response to additional fertilizer-N while those with lower INS showed a strong yield response (Fig. 2b). Negative yield responses were observed in five trials (SOC-114 to SOC-156; Fig. 2b). No significant positive yield response to N application was observed in trials between SOC-114 and SOC-176. Both the low (SOC-27 to SOC-100) and high (SOC-232) ends of the gradient exhibited a yield response to N application (Fig. 3).

Soil Incubations

In the anaerobic incubation studies, NH$_4^+$ concentrations increased initially for all soils. In general, peak NH$_4^+$ concentrations increased as SOC content increased, with the smallest peak NH$_4^+$ concentration (26 mg kg$^{-1}$) observed in the SOC-27 soil and the greatest peak NH$_4^+$ concentration (221 mg kg$^{-1}$) observed in the SOC-176 soil (Fig. 4). Peak NH$_4^+$ concentrations occurred earlier in the incubations as SOC increased, shifting from 49 d for the SOC-57 soil to 14 d for the SOC-232 soil. Across soils, NH$_4^+$ concen-

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**Table 2.** Fixed effects parameter estimates from a mixed-effects model of rice N uptake by soil organic C (SOC) content and fertilizer-N applied for 10 N rate trials and 15 N omission plots in the Sacramento–San Joaquin Delta from 2011 to 2013.

<table>
<thead>
<tr>
<th>Fixed effect</th>
<th>Estimate</th>
<th>95% Bootstrapped confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>94.5</td>
<td>(59.8, 126.2)</td>
</tr>
<tr>
<td>Fertilizer-N uptake efficiency</td>
<td>0.38</td>
<td>(0.28, 0.49)</td>
</tr>
<tr>
<td>SOC</td>
<td>1.44</td>
<td>(0.92, 2.04)</td>
</tr>
<tr>
<td>SOC$^2$</td>
<td>-0.0055</td>
<td>(-0.0080, -0.0031)</td>
</tr>
<tr>
<td>Residual error</td>
<td>21.6</td>
<td>(19.3, 24.2)</td>
</tr>
<tr>
<td>$R^2$ marginal</td>
<td>0.44</td>
<td>–</td>
</tr>
<tr>
<td>$R^2$ conditional</td>
<td>0.81</td>
<td>–</td>
</tr>
</tbody>
</table>
trations decreased following these peak levels, with an increasing rate of decline as SOC increased. For example, NH$_4^+$ concentrations fell in the SOC-57 soil by 77 d while NH$_4^+$ concentrations fell by 28 d in the SOC-232 soil (Fig. 4). For all soils, once NH$_4^+$ concentrations decreased, they remained low for the remaining duration of the incubation. Throughout the anaerobic incubation, nitrate levels were low (less than 0.1 mg NO$_3^-$ L$^{-1}$) or zero in the soil extracts (data not shown).

**DISCUSSION**

**Indigenous N Supply Related to Soil Organic C**

Contrary to previous reports of there being little or no association between the INS and SOC in rice systems (Cassman et al., 1996a; Dobermann et al., 2003), results from this study show a strong relationship between SOC and the INS (Fig. 1). The observation of this effect was the result of extending the range of SOC under consideration, though the estimated linear effect (1.44 kg N ha$^{-1}$ for every g kg$^{-1}$ increase in SOC) is comparable with that measured by Dobermann et al. (2003). On the basis of only this estimate, the impact of SOC on the INS in the context of typical rice paddy soils (with between 0 and 20 g SOC kg$^{-1}$) is relatively small, but becomes more important in the context of rice grown on organic soils. The contribution of SOC to the INS becomes sufficient by 110 g SOC kg$^{-1}$ to supply the entire N requirements of the rice crop.

Contrary to the hypothesis that the INS would increase linearly with SOC across the entire range of SOC, these findings show that the INS first increased and then decreased as soils increase in SOC. At 131 g SOC kg$^{-1}$ the INS begins to drop and at 232 g SOC kg$^{-1}$ had decreased to the point where rice became substantially N limited. This relationship between the INS and SOC is described here as concave quadratic (Fig. 1) and is con-
consistent with both the observed N yield response across the SOC gradient (Fig. 3) and published reports of N deficiency of rice cultivated on soils with greater than 300 g SOM kg$^{-1}$ (roughly greater than 160 g SOC kg$^{-1}$) (Ponnamperuma, 1985; Lantin et al., 1990; Driessen and Soeprapohardjo, 1974). Despite consistency with previous work, this study has relatively poor data resolution below 50 g SOC kg$^{-1}$ and above 170 g SOC kg$^{-1}$. Since the estimate for the quadratic trend is heavily influenced by data at either end of the range, the estimate of the quadratic effect produced here could be further improved by additional data from rice grown on soils with high SOC.

The anaerobic soil incubations showed differences among soils ex situ that may explain why the INS would decrease in high SOC soils rather than continuing to increase. The high SOC soils had relatively large amounts of mineralized N for a short period, while the lower SOC soils had smaller amounts of mineralized N but over a longer interval (Fig. 4). If this pattern is mirrored in the field, N mineralized as high SOC soils are flooded might be available for a short interval early in the season when rice plant N uptake capacity is limited (Shimono and Bunce, 2009; Norman et al., 2003; Peng and Cassman, 1998; Youngdahl et al., 1982; Moore et al., 1981). Under this conceptualization, the INS is low in low SOC soils because there is low mineralization potential; the INS is high in the intermediate range because there is medium mineralization potential and mineralized N remains available for plant uptake; the INS is low in high SOC soils because mineralized N decreases before plants can accumulate it.

Although this study cannot definitively speak to the cause of the decrease in N concentrations observed in the incubations, immobilization is the most plausible explanation. Assuming the incubation is a closed system, immobilization and gaseous N losses are the most likely mechanisms that could be responsible for decreased N concentrations over time. The primary gaseous N loss pathway in an enclosed anaerobic incubation would be ammonia oxidation (Zhu et al., 2013), yet the lack of NO$_3^-$ in solution throughout the incubation combined with continuous anaerobic conditions make it unlikely that this pathway was a significant source of N losses. Therefore, immobilization is the most probable explanation for the patterns observed in the incubations.

Differences in immobilization potential among soils provides a reasonable explanation for the decrease in NH$_4^+$ in the incubation and decreased N availability in soils high in SOC. Published reports have observed strong N mineralization following drying and rewetting of organic soils but low or no N mineralization in continuously wet organic soils (Ponnamperuma, 1985; Sahrawat, 1981), corroborating that flooded organic soils may have strong immobilization potential.
Unexplained Variability in the Indigenous N Supply

Consistent with other reports on the INS, there was high variability in the INS among sites (Table 3; Fig. 1) (Cassman et al., 1996a; Dobermann et al., 2003; Wang et al., 2012). This is evident in the large proportion of the overall variability that resides in the random components of the mixed-effects model, as seen in the difference between the $R^2_{\text{marginal}}$ (0.44) and $R^2_{\text{conditional}}$ (0.81). Given that the INS is an aggregate of contributions from multiple sources that are potentially sensitive to site differences (e.g., straw decomposition, nonsymbiotic N fixation, irrigation N, atmospheric N deposition, and SOC mineralization), this variability is expected. Here, all non-SOC contributions to the INS fell under the intercept term of the model with the overall intercept estimated to be 94.5 kg N ha$^{-1}$ (Table 2). Although there is substantial uncertainty in this estimate, the lower boundary of the confidence interval is far from zero (60 kg N ha$^{-1}$). This suggests that combined, these other sources provide a substantial contribution to the INS across all soils.

The magnitude of non-SOC contributions to the INS observed at several sites in this study (overall intercept plus site random intercept) was greater than reported for mineral soils (30 to 90 kg N ha$^{-1}$ [Cassman et al., 1996a; Dobermann et al., 2003; Linquist et al., 2009; Wang et al., 2012]). Previous work at Twitchell Island has suggested that there may be a substantial pool of below-ground NH$_4^+$ associated with pore water that could explain some of this variation. Although pore water was monitored in 2013 and differences were observed in 60-cm pore-water N concentrations between sites, these measurements were not correlated with either observed N uptake or SOC (data not shown). There is no direct evidence in this study that pore-water N contributed to plant N uptake, though the consequence of such a contribution would be higher estimates for site and overall intercepts.

N Recommendations Based on Soil Organic C

Observed grain yields compare favorably with California state averages for medium grain rice (9600 kg ha$^{-1}$ for 2012 [California Agriculture Statistics Service, 2013]). Average N application recommendations to achieve similar yields on mineral soils are typically between 150 and 170 kg N ha$^{-1}$ (Linquist et al., 2009; Mutters et al., 2013).

Most organic soils will require less N fertilizer than mineral soil to produce comparable grain yields. Given that soils in the range of 114 to 176 g SOC kg$^{-1}$ showed no significant positive yield response to N fertilizer (Fig. 3), and given that the INS in that same range averaged roughly 189 kg N ha$^{-1}$ (Fig. 1), N was not a yield-limiting factor for soils between 114 and 176 g SOC kg$^{-1}$. Furthermore, the negative yield responses to added N observed in five of the trials in this same range (Fig. 2b) may be due to grain blanking caused by excessive N (Board and Peterson, 1980). This suggests that applying additional N to these soils may actually be detrimental to achieving high yields.

These results indicate that recommended N application rates for rice should depend on SOC values. In the range from 110 to 170 g SOC kg$^{-1}$, no additional N application should be required. Below 110 g SOC kg$^{-1}$ application rates should decrease from 150 to 170 kg N ha$^{-1}$ to zero as SOC increases (Fig. 3). For soils with greater than 170 g SOC kg$^{-1}$, N application rates should increase from zero as SOC increases, though optimum N rates for soils with greater than 170 g SOC kg$^{-1}$ is unknown at this time.

Estimated fertilizer-N uptake efficiencies were low across sites, averaging 38% (Table 2), with only three sites exhibiting greater than 50% fertilizer-N uptake efficiency (Table 3). Although few N rate trials showed evidence of N limitations (with most fertilizer-N uptake between 110 and 170 g SOC kg$^{-1}$ likely being luxury consumption), low fertilizer-N uptake efficiency on soils responsive to fertilizer-N suggests room for further improvement. Increasing fertilizer-N uptake efficiency would allow for further reduced N application rates.

CONCLUSIONS

The hypothesis that the INS would increase linearly in response to increasing SOC was rejected. Results show the INS is predicted by SOC following a quadratic trend, but high unexplained variability remains. The ratio of the effect of SOC to the unexplained variability (i.e., signal to noise) is low in the context of soils on which rice is typically grown (less than 20 g SOC kg$^{-1}$). However, the impact of SOC becomes more noteworthy as SOC increases, becoming substantial for rice grown on highly organic soils. Nitrogen rate recommendations for organic soils should therefore consider SOC content. Starting from current N rate recommendations for soils with low SOC, rates should decrease to little or no additional N for soils in the range of 100 to 170 g SOC kg$^{-1}$ and increase again for soils with greater than 170 g SOC kg$^{-1}$.

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